

bolic data are taken interacts complexly with the size and shape of the organisms (for example, small animals are influenced more by wind, in terms of convective heat transfer, than large animals). Thus, the regression model (Eq. 1) may not be an appropriate tool for comparing metabolic responses of different species of homeotherms (or even different-sized homeotherms of the same species), and it is certainly inappropriate as a predictive model of the metabolic responses of homeotherms in natural environments (4).

Kleiber has applied "an especially simple form of Fourier's law to the heat flow in the body of homeothermic animals schematized as a core with a constant temperature T_b , surrounded by an insulating layer with heat conductivity λ , thickness L , surface area S , and surface temperature T_s " [(1); see also (5)]:

$$\frac{dQ}{dt} = \lambda \frac{S}{L} (T_b - T_s) \quad (2)$$

where dQ/dt is the instantaneous heat flow from the animal. Kleiber also noted that Eq. 2 is used by architects and engineers to calculate the heat conducted from houses, but its use in physiological publications has been rare (1). Actually, Eq. 2 is a special case of Fourier's equation (6) that is well suited for calculations of heat transfer through flat slabs (such as the walls of houses), but possibly inappropriate for descriptions of heat transfer from animals. For example, heat transfer through a flat slab is conducted through the same cross-sectional area throughout the thickness of the slab, but heat transfer from a roughly cylindrical animal is transferred from a core, with a definable surface area, to the outer surface of the insulating "shell," where the surface area is relatively larger. Therefore, heat flow through the insulating layer of animals is not linear with respect to position in the insulating layer, as suggested by Kleiber's approximation (Eq. 2) of the Fourier equation.

For the sake of semantic clarity, historical truth, and mechanistic accuracy, Kleiber's distinctions between Fourier's law and Newton's law are very important. However, it is perhaps even more important for biologists to know the limitations of the equations they have for so long called Newton's or Fourier's law (4, 7).

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References and Notes

1. M. Kleiber, *Science* **178**, 1283 (1972).
2. W. P. Porter and D. M. Gates, *Ecol. Monogr.* **39**, 245 (1969).
3. When air temperature is the only environmental variable in the driving function of these equations, it is implicitly assumed that the thermal radiative environment and the environment implicated in conductive heat transfer from the animal are at the same temperature as air. This assumption may be in error by as much as 30°C under natural conditions. Evaporative heat loss cannot be properly accounted for in Eq. 1, because the driving function for evaporative heat loss must include relative humidity as well as environmental temperature.
4. C. R. Tracy, *BioScience* **22**, 656 (1972).
5. M. Kleiber, *Hilgardia* **6**, 323 (1932); *The Fire of Life* (Wiley, New York, 1961).
6. Fourier's law is $dQ/dt = -kA(dT/dx)$, where dQ/dt is the instantaneous heat flux across the area A , k is the thermal conductivity, and dT/dx is the temperature gradient at A [see J. P. Holman, *Heat Transfer* (McGraw-Hill, New York, 1968), p. 2].
7. T. H. Strunk, *J. Theor. Biol.* **33**, 35 (1971).

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I appreciate Tracy's recognition and am sorry that Strunk finds that my report has not helped to clarify the situation. He blames me for not discussing the equation $M = C(T - T_a)$, which states that the metabolic rate of animals is proportional to the difference between body temperature and

ambient temperature. Newton's law deals with temperature loss and Fourier's law with heat flow; neither expresses metabolic rate and I was justified in avoiding an unnecessary complication in my report. Since both commentators brought up the metabolic rate, I can add that the equation above applies to homeotherms only in an ambient temperature below the lower critical temperature, when the metabolic rate changes as a part of "chemical temperature regulation" (1). When the ambient temperature rises to the thermoneutral zone the metabolic rate becomes independent of changes in ambient temperature. I have discussed this special problem elsewhere (2).

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1. M. Rubner, *Gesetze des Energieverbrauchs bei der Ernährung* (Deuticke, Leipzig, 1902).
2. M. Kleiber, *The Fire of Life* (Wiley, New York, 1961), pp. 161-166; *J. Theor. Biol.* **37**, 139 (1972).

17 May 1973

Serious Contaminant in "Ultra Pure" Grades of Sucrose

In the course of our studies we have discovered that several lots of the "Ultra Pure enzyme-grade" (Nos. X3822 and X3233) and "RNase-free grade" sucrose (No. X3927) supplied by Schwarz/Mann contains a contaminant that may preclude its use in certain density gradient applications. When subcellular particles such as polyribosomes are purified by zonal centrifugation on gradients prepared with this sucrose, and then RNA is extracted from the particles by a conventional procedure such as that in which sodium dodecyl sulfate, chloroform, and phenol are used, the contaminant is isolated together with the RNA. The contaminant, which is insoluble in 70 percent ethanol, is also relatively insoluble at the low salt concentrations ($\leq 0.1M$) normally used to dissolve RNA, producing a slight turbidity which can interfere with ultraviolet absorption measurements. An even more serious consequence of the contaminant is that its presence in RNA preparations causes an appreciable amount of nonspecific binding of the RNA to both Millipore and polyuridylic acid-impregnated glass fiber filters which are now commonly used in assays of RNA containing polyadenylic acid. Fortunately, the contaminant is readily soluble in 1M NaCl,

and, thus, if one washes the RNA precipitates with 1M NaCl before dissolving them in the low salt solution, one can eliminate the contaminant, and obtain RNA that gives reliable results with the Millipore or polyuridylic acid-glass fiber filter binding assays. Of course, there is an unavoidable loss of low-molecular-weight RNA by this procedure.

The contaminant was not found in the grade 1, crystalline sucrose supplied by another company. Its presence in any particular sample of sucrose can be demonstrated by adding an equal volume of ethanol to a 45 percent solution of the sucrose. The solution, after standing at room temperature, will exhibit readily visible turbidity within 15 minutes if the contaminant is present.

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Schwarz/Mann offers Ultra Pure Sucrose, No. 90-9530, as a special grade, free of ribonuclease activity, for sucrose density gradient centrifugation of RNA preparations. Our assay procedure for ribonuclease calls for 18 hours of incubation with RNA at 37°C and

measurement of the production of acid-soluble ultraviolet-absorbing material. If any such material is released, the lot is not offered for sale. In addition, heavy metals may not exceed 5 parts per million. All lots sold by Schwarz/Mann, including the one referred to by Greenberg *et al.* (1), meet these specifications. There is no ribonuclease, and heavy metals are less than 5 parts per million.

We apply these strict specifications because of the many varied and critical procedures in which this sucrose may be used. The Schwarz/Mann density gradient sucrose used by Greenberg *et al.* came from a lot purchased in 1972 (lot 3927). Using his procedure, we confirmed the turbidity and isolated the material responsible for it. Our investigations showed the presence of a polysaccharide, possibly amylose or cellulose. This conclusion was drawn from the fact that the material (i) has no appreciable ultraviolet absorbance at 260 or 280 nm, (ii) has an infrared spectrum similar to that of saccharides, (iii) no amino acid content (except for small amounts of methionine), and (iv) responds positively to classic starch tests.

As a result, all lots of Schwarz/Mann density gradient sucrose sold from October 1972 through the present (Y1205, Y1229, Y1488, Y1562, Y1668, Y1786, Y1862, and Y3102) were reassayed by the alcohol-water test (1). All lots were found free of this material. In addition, this test will be included in assaying all Schwarz/Mann density gradient sucrose prior to release for sale. The enzyme grade mentioned by Greenberg *et al.* is not recommended for use in density gradients.

It is regrettable when any researcher finds one lot of our sucrose unsuccessful in his procedure. When this happens, Schwarz/Mann offers to replace the material involved. If the procedure has merit, Schwarz/Mann includes the assay in its quality control analysis for the product. A product evolves as its specifications are adapted to the changing needs of research, and we invite your correspondence as to your specification requirements for sucrose or any other Schwarz/Mann product.

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1. J. R. Greenberg, D. E. Kelley, R. P. Perry, K. D. Tartof, *Science* **181**, 186 (1973).

7 May 1973

13 JULY 1973

Somatic Evoked Response Recording: An Adequate Test of Deafferentation?

Cohn *et al.* (1) recorded somatic averaged evoked responses from the scalp of monkeys (*Macaca mulatta*) and a baboon (*Papio*) to test for the completeness of forelimb deafferentation resulting from dorsal rhizotomy. No responses were seen after rhizotomy. The authors concluded that all somatic input from the limb to cerebral cortex was eliminated and that their procedure "constitutes a new critical determinant of the functional effectiveness of the experimental surgery." In our opinion these conclusions are not justified by the data presented, and cannot be by the technique used, for several reasons.

It is tenuous to conclude on the basis of scalp recording that cortical evoked activity is absent. Scalp recordings have an inferior signal-to-noise ratio relative to cortical recording because

of significant attenuation of the response by the skull and scalp, and because of the introduction of electrical "noise" from extracerebral sources. Cohn *et al.* used Michel clips to record from the scalp. This is not a commonly used scalp electrode and the quality of the records obtained with its use has not been demonstrated. Recordings of better quality can be obtained in animals directly from the surface of the brain and are more appropriate when attempting to demonstrate the absence of an evoked response. Figure 1 shows somatic evoked responses recorded directly from the cortical surface in four chronically implanted *M. mulatta*; a large primary positive wave with a peak latency of about 14 msec is followed in most animals by a positive-negative sequence at about 40 and 60 msec. A scalp recorded response of similar waveform (but smaller in amplitude owing to the attenuation noted above) is seen also in the *Cebus* monkey (2). The evoked responses shown by Cohn *et al.* do not appear to resemble these responses, although comparison with their figure is difficult because evoked response component resolution is inadequate and response polarity is not indicated. This lack of characteristic waveform in their responses from the intact animal could result from inaccurate location of the scalp electrode with respect to the primary cortical sensory area. This suggestion is further supported by the fact that their responses from an intact animal are hardly larger than the presumed spontaneous activity recorded from the rhizotomized animal. For any or all of the above reasons, the lack of a response in their operated animals could indicate merely that the rhizotomy produced a partial deafferentation which reduced the cortical response to a level undetectable by scalp recordings.

Although in man, and perhaps in other primates, somatic evoked responses of the type recorded by Cohn *et al.* are mediated mainly by the dorsal column-medial lemniscal afferent pathway (3), the anterolateral tracts of the spinal cord also provide input to various cerebral regions, for example, the midbrain reticular formation (4, 5) and the thalamic nucleus centre median (6). The activity thus evoked requires higher stimulus intensities (4, 5) and is potentiated by chloralose anesthesia

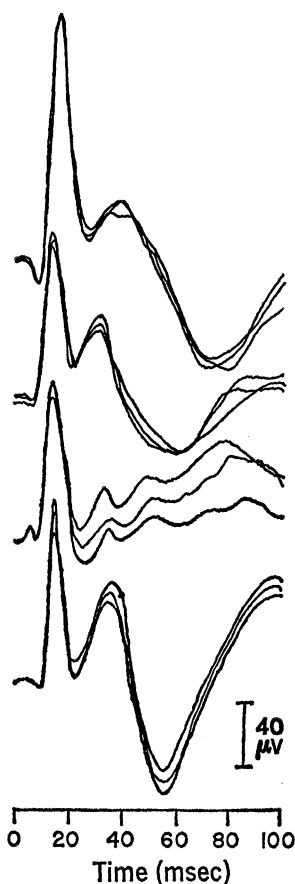


Fig. 1. Averaged somatic evoked responses in four *M. mulatta*. Responses were recorded epidurally from primary somatic cortex in the postcentral gyrus, to percutaneous stimulation of the contralateral median nerve. Each trace is the average of 16 responses; for each animal three averaged responses are superimposed to indicate variability. Positivity at the active electrode is upward referential to an electrode in bone overlying frontal sinus.